Role of Renal Nerves in Mediating the Blunted Natriuretic Response to Acute Saline Load in Obese Dogs

Salah Kassab, Tatsuya Kato, F. Clayton Wilkins, Leland Mizelle, and Joey P. Granger

We previously reported that the natriuretic response to an acute sodium load is markedly attenuated in obese dogs. Since obesity is associated with enhanced sympathetic nervous activity, the purpose of this study was to test the hypothesis that obese animals have a reduced ability to excrete a sodium load as a result of abnormal renal nerve function. To test this hypothesis, we determined the effects of an acute sodium load (100 mEq NaCl given as isotonic saline over 60 min) in lean (19.8 ± 1.0 kg) and obese (25.8 ± 1.1 kg) dogs. Two surgically designed hemibladders with indwelling catheters were used to collect urine from innervated (INN) and denervated (DNX) kidneys of the same dog. Arterial pressure averaged 99 ± 3 mm Hg in the obese dogs and 90 ± 3 mm Hg in the lean dogs. In response to the saline loading in lean dogs, sodium excretion (UNaV) increased from 31.0 ± 7.8 to 145.6 ± 25.9 μEq/min in the INN kidneys and from 43.1 ± 10.6 to 151.1 ± 28.4 μEq/min in the DNX kidneys. In contrast, UNaV in obese dogs increased from 10.3 ± 3.0 to 110.1 ± 25.5 μEq/min in the INN kidneys and from 24.4 ± 2.7 to 106.1 ± 20.6 μEq/min in the DNX kidneys. Cumulative sodium excretory response to sodium loading was significantly lower in the obese dogs. In addition, there was no difference in the cumulative UNaV response between the INN (8.4 ± 2.2 mEq) and DNX kidneys (9.1 ± 2.3 mEq) of obese dogs. These data indicate that the natriuretic response to an acute saline loading is markedly attenuated in obese dogs. Furthermore, factors other than renal nerves play a role in this abnormal response.

KEY WORDS: Obesity, renal sympathetic nervous system, hypertension, dog.
response to an acute saline load is significantly attenuated in obese dogs. However, the exact mechanism responsible for the blunted volume expansion induced natriuresis in obese dogs is unknown. Because obesity is associated with enhanced sympathetic nervous activity, we proposed that obese animals may have a reduced ability to excrete a sodium load as a result of abnormal renal sympathetic nerve function. Consistent with this hypothesis are previous findings indicating that the attenuated renal excretory response to an acute oral or intravenous saline load in several sodium-retaining models is significantly improved by prior bilateral renal denervation. However, the extrapolation of this mechanism to explain the blunted natriuresis in obesity is unclear, since a recent study failed to demonstrate a role for the renal nerves in the blunted renal response to an acute saline load in obese Zucker rats.

The purpose of this study was to determine the role of renal nerves in the attenuated renal excretory response to an acute saline load in obese dogs. To test this hypothesis, we studied the changes in renal hemodynamics and \( U_{\text{NaV}} \) in separate innervated (INN) and denervated (DNX) kidneys simultaneously within the same dog. This model is extremely sensitive in detecting any effect of the renal nerves, because each kidney is exposed to the same arterial pressure and same level of circulating hormones. Any difference in renal excretion between INN and DNX kidneys can then be attributed to a direct or an indirect effect of the renal nerves.

**METHODS**

These experiments were conducted on female mongrel dogs (n = 12). Surgery and care of animals in this study were conducted according to National Institutes of Health guidelines for Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee at the University of Mississippi Medical Center. All surgical procedures were performed under general anesthesia using pentobarbital sodium (30 mg/kg, intravenously). Each dog underwent surgery for implantation of chronic femoral arterial and venous catheters. The catheters were then tunneled subcutaneously and exteriorized at the neck to allow easy access to blood sampling, infusions, and measurement of arterial blood pressure. At the same setting, the left kidney was exposed through a dorsolateral incision in the flank, the renal hilum was exposed, and all the nerves running along the renal artery and vein were carefully dissected and removed. In addition, the adventitia was stripped from the vessels and painted with a solution of 10% phenol in absolute ethanol. Kidneys were accepted as being denervated only if tissue catecholamines were depleted. Our denervation technique reduced renal tissue norepinephrine concentrations in denervated kidneys to < 10% of norepinephrine concentrations of innervated kidneys.

Approximately 7 days later, the dogs were anesthetized again for the formation of two hemibladders as previously described. Briefly, the bladder was exposed through a midline suprapubic incision and the urethra was isolated. The distal end of the urethra was ligated and divided close to the bladder neck. The urinary bladder was hemisected evenly using a GIA 90 Premium surgical stapler (United States Surgical Corp., Norwalk, CT), and then closed with very tight sutures starting from the distal end and finishing in the trigone between the ureteric orifices. A small opening was created at the base and a urine catheter was inserted and secured in place by purse string sutures. Each hemibladder was then checked for any leakage by injection of saline. The catheters were then exteriorized across the flanks and connected to 1 L urine bags that were protected by rigid plastic tubes contained in a jacket worn by the dog. This preparation allowed the collection of urine from each kidney separately.

Immediately after surgery, the dogs were given butorphanol tartrate (0.3 mg/kg, intramuscularly) to allow a painless postoperative period, and then left to recover from surgery for at least 7 days, under a cover of antibiotics in the form of a twice-daily dose of oral amoxicillin (500 mg) and a sulfamethoxazole/trimethoprim (400 mg/80 mg) combination.

Six dogs were made obese by maintaining them for 5 weeks on a high-fat diet of cooked beef fat (0.7 to 0.9 kg/day) in addition to their regular sodium-deficient diet that contained about 7 mEq of sodium (H/D Hills Pet Products, Topeka, KS). The other six (control) dogs were maintained on their regular 2 cans of sodium-deficient dog food (H/D Hills Pet Products, Topeka, KS).

**Experimental Protocol** After at least 7 days of recovery, dogs were housed in individual metabolic cages in an air-conditioned room with a 12-h light/dark cycle and fitted with harnesses containing a pressure transducer mounted at the level of the heart. Isotonic saline was continuously infused intravenously at a fixed rate of 400 mL/day to maintain the total sodium intake at approximately 65 mEq/day, including the sodium (7 mEq/day) provided in food. A period of 10 to 14 days was allowed for the dogs to achieve a stabilization of hemodynamic measurements and renal excretory function.

On the day of the experiment, \( ^{125}\text{I}-\text{iothalamate} \) (Glofil, Isotex Diagnostics, Friendswood, TX) and \( ^{131}\text{I}-\text{iodohippurate} \) (CIS, Bedford, MA) were added to the saline infusion for measurement of glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), respectively. A priming dose of 0.45 \( \mu \text{Ci} / \text{kg} \) of iothalamate..
and 1.0 μCi/kg of iodohippurate were given, followed by a sustained infusion of 0.005 and 0.010 μCi/kg/min of iothalamate and iodohippurate, respectively.

Animals were allowed a 1-h equilibration period prior to the beginning of the experiment. Ten minutes before urine collection, the bladders were flushed with distilled water and arterial catheters were flushed with 5% dextrose solution. After obtaining two 20-min control clearances, the animals were infused with 660 mL of isotonic saline (100 mEq NaCl) over a 60-min period. Three clearances were obtained during the period of infusion followed by four postinfusion clearances of 20 min each. Blood samples were taken during the midpoint of each urine collection. During the experiment, the mean arterial pressure (MAP) and heart rate were monitored continuously using a Statham pressure transducer, and the analog signals were sent to a digital computer to be analyzed. The computer program was adjusted to calculate the average blood pressure and heart rate values recorded every 20 min.

Plasma and urine sodium and potassium concentrations were determined by flame photometry (IL-943, Instrumentation Laboratory, Lexington, MA). The levels of radioactivity from 125I-iothalamate and 131I-iodohippurate in the plasma and urine were used to calculate GFR and RPF, respectively.

At the end of the experiment, animals were euthanized with intravenous potassium chloride solution under pentobarbital anesthesia. The kidneys were taken out immediately and homogenized in 0.1 perchloric acid. The homogenate was centrifuged at 22,000 rpm and the supernatant was stored at −70°C until assayed. Renal tissue norepinephrine concentration was measured in INN and DNX kidneys using high-performance liquid chromatography with electrochemical detection as previously described.14 The kidneys were accepted as being DNX only when renal tissue catecholamines were markedly depleted. In this study, renal tissue catecholamine concentration in the DNX kidneys was < 10% that in the INN kidneys.

Statistical Analysis Average control values were compared with those after volume expansion by using analysis of variance for repeated measures and Dunnett’s t test. Comparison between INN and DNX kidneys within each group was made by using paired t tests. All data are expressed as mean ± SE. A P < .05 was considered statistically significant.

RESULTS

Feeding a high-fat diet for 5 weeks increased the body weight of the obese group significantly from 17.9 ± 1.0 kg to 25.8 ± 1.1 kg. The average body weight of the lean control group was 19.8 ± 1.0 kg. The mean arterial pressure (MAP) in the lean dogs was 90 ± 3 mm Hg, while it was 99 ± 3 mm Hg in the obese dogs, a difference that did not reach statistical significance. The heart rate was significantly higher in obese dogs compared with lean control dogs. Heart rate in lean dogs averaged 90 ± 7 beats/min, and in obese dogs 140 ± 10 beats/min. As shown in Figure 1, both heart rate and arterial pressure did not change significantly in response to volume expansion in either lean or obese dogs.

Figure 2 depicts the sodium excretory response to volume expansion in the INN and DNX kidneys of lean and obese dogs. Acute volume expansion with 660 mL of isotonic NaCl for 60 min resulted in a significant increase in urinary U NaV in both lean and obese dogs. However, UNaV was significantly attenuated in obese compared with lean dogs. Urinary UNaV in lean dogs increased from 31.0 ± 7.8 to 145.6 ± 25.9 μEq/min in the INN kidneys, and from 43.1 ± 10.6 to 151.1 ± 28.4 μEq/min in the DNX kidneys. In contrast, UNaV in obese dogs increased from 10.3 ± 3.0 to 110.1 ± 25.5 μEq/min in the INN kidneys and from 24.4 ± 2.7 to 106.1 ± 20.6 μEq/min in the DNX kidneys. Under baseline conditions, there was a significant difference in UNaV between the INN and DNX kidneys of obese dogs. However, the difference in UNaV between INN and DNX kidneys of lean dogs was not significant.

Cumulative UNaV in obese dogs was 37% lower than in lean dogs (Figure 2). Cumulative UNaV in lean dogs was 14.3 ± 1.9 mEq in the INN kidneys and 15.9 ± 2.1 mEq in the DNX kidneys. However, cumulative UNaV in obese dogs was 8.4 ± 2.2 mEq in the INN kidneys and 9.1 ± 2.3 mEq in the DNX kidneys. There was no significant difference in cumulative UNaV between INN and DNX kidneys of both groups.

The urine flow rate (UFR) significantly increased after saline infusion in both lean and obese dogs (Figure 3). In lean dogs, UFR increased from 0.71 ± 0.1 to 1.4 ± 0.2 mL/min in the INN kidneys, and from 0.79 ± 0.07 to 1.49 ± 0.14 mL/min in the DNX kidneys. In obese dogs, UFR increased from 0.66 ± 0.07 to 1.5 ± 0.24 mL/min in the INN kidneys, and from 0.74 ± 0.1 to 1.52 ± 0.39 mL/min in the DNX kidneys. There was no significant difference in UFR between lean and obese dogs or between the INN and DNX kidneys of both groups.

Figure 4 illustrates the renal hemodynamic responses to volume expansion in the INN and DNX kidneys of lean and obese dogs. ERPF increased significantly in both lean and obese dogs and tended to decrease toward normal after 80 min. In lean dogs, ERPF increased from 85.8 ± 8.9 to 98.0 ± 7.2 mL/min in the INN kidneys and from 83.5 ± 8.1 to 96 ± 9.3 mL/min in the DNX kidneys. In obese dogs, ERPF increased from 90.5 ± 11.1 to 109.6 ± 14.7 mL/min in the INN kidneys and from 93.9 ± 12.5 to 114.1 ± 15.0 mL/min in the DNX kidneys. GFR also tran-
FIGURE 1. Effect of acute saline loading on mean arterial pressure (MAP) and heart rate of lean and obese dogs.

siently increased in both lean and obese dogs. In lean dogs, GFR increased from 31.9 ± 2.3 to 39.5 ± 2.3 mL/min in the INN kidneys and from 31.8 ± 2.8 to 43.2 ± 4.9 mL/min in the DNX kidneys. In obese dogs, GFR increased from 28.6 ± 3.1 to 36.4 ± 4.1 mL/min in the INN kidneys and from 28.4 ± 2.6 to 34.9 ± 4.2 mL/min in the DNX kidneys. There was no significant difference in GFR or ERPF between lean and obese dogs or between the INN and DNX kidneys of either group.

DISCUSSION

The present study was designed specifically to analyze the role of renal nerves in the blunted natriuretic response to an acute saline load in obese dogs. The changes in renal hemodynamics and sodium excretory responses to volume expansion were examined in separate INN and DNX kidneys simultaneously within the same dog. The results of the present study are in agreement with our previous findings that the sodium excretory responses to acute sodium load is markedly attenuated in obese dogs compared with lean controls. Furthermore, our study demonstrates that renal denervation does not significantly alter the sodium excretory response to an acute saline load in obese dogs.

Feeding a high-fat diet to dogs for 5 weeks increased body weight by approximately 40% to 45%. Associated with increased body weight were significant increases in heart rate of 55% and small but insignificant increases in arterial pressure. Although these findings are consistent with previous findings from
our laboratory and others,\textsuperscript{3,6,15} the arterial pressure increase was less than previously observed. It is possible that the unilateral renal denervation may have attenuated the sodium retention and virtually abolished the hypertension during the 5-week period of the high-fat diet. Supporting this notion are the results from a preliminary study from our laboratory indicating that unilateral renal denervation resulted in less sodium retention in the DNX kidneys than in the INN kidneys during the development of obesity-induced hypertension.\textsuperscript{16} Whether the improvement of renal sodium excretion and reduction in blood pressure in the renal denervated obese dogs is due to removal of efferent or afferent nerves is unclear. However, preliminary studies from our laboratory indicate that specific renal deafferentation does not affect the sodium retention or the development of hypertension in obese dogs.\textsuperscript{13}

Previous studies have indicated that the development of obesity-induced hypertension may be related to an abnormality in the regulation of sodium homeostasis.\textsuperscript{5,15} We have recently demonstrated that the natriuretic response to an acute saline load is significantly attenuated in obese dogs.\textsuperscript{6} The mechanisms responsible for the blunted natriuretic response to an acute saline load in obesity, however, have not been determined. A possible role of the renal nerves in the sodium retaining abnormality of obesity has been inferred from previous studies that demonstrated an increased sympathetic nervous system activity in obe-

\textbf{FIGURE 2.} Effect of acute saline expansion on urinary sodium excretion (UNaV) (upper panel) and cumulative sodium excretion (lower panel) in the innervated (INN) and denervated (DNX) kidneys of lean and obese dogs. Values are mean ± SE.
Further support derives from studies indicating that the attenuated renal excretory response to an acute oral or intravenous saline load in sodium-retaining models, such as congestive heart failure, liver cirrhosis, and nephrotic syndrome, is significantly improved by prior bilateral renal denervation. The results of our experiment suggest, however, that removal of the renal nerves in the dog model of obesity does not improve the renal excretory response to an acute saline load. Although renal denervation improved sodium excretion under basal conditions in the obese dogs, the sodium excretory response to saline was the same between the INN and DNX kidneys in the obese dogs. Consistent with our findings are the results of a recent study that failed to demonstrate a role of renal nerves in the blunted renal excretory response to an acute saline load in obese Zucker rats. Thus, although renal nerves do play a role in attenuating the natriuretic responses to a saline load in certain sodium retaining states, such as in congestive heart failure, they do not appear to play a significant role in the attenuated response observed in obesity.

It appears unlikely that the differences in systemic or renal hemodynamics are responsible for the attenuated natriuretic response in obese dogs. Arterial pressure did not increase in response to volume expansion in either the obese or control dogs. Furthermore, volume expansion increased GFR and ERPF similarly in both lean and
obese dogs. There was also no significant difference in GFR or ERPF between the INN and DNX kidneys in response to acute saline loading. These results would indicate that the blunted natriuretic response to an acute saline loading in obese dogs may be due to abnormalities in renal tubular reabsorption of sodium.

The results of the present study indicate that abnormalities of nonneural sodium regulatory mechanisms may play a role in the blunted natriuretic response to an acute saline load. Although the exact mechanism involved in this response is unclear, there are several possibilities. One mechanism that has been recently proposed is that obese animals have an abnormality in atrial natriuretic factor (ANF) release in response to an acute saline load. Supporting this theory are the results of a recent preliminary study indicating that obese humans do not have enhanced ANF release during extracellular volume expansion.\(^{20}\) Another possible mechanism may involve the renin-angiotensin system. Plasma renin activity is elevated in human and animal models of obesity.\(^{8,15,19}\) An inability to suppress the plasma renin activity in obese dogs could also account for the abnormal renal handling of sodium. We have previously reported that saline volume expansion suppressed plasma renin activity in obese and lean dogs.\(^{6}\) However, the level of plasma renin activity was significantly higher in obese dogs under basal conditions and in response to volume expansion. The quantitative importance of these and other possible mechanisms in the blunted natriuretic response to acute saline loading in obesity is unknown and will require further investigation.

**FIGURE 4.** Renal hemodynamic responses to an acute saline loading in lean and obese dogs. GFR, glomerular filtration rate; RPF, renal plasma flow; other abbreviations as in Figure 2.
The sodium load used in this experiment was approximately 100 mEq NaCl given as isotonic saline over a 60-min period. This quantity of NaCl is approximately 2 to 3 times the normal daily sodium intake of dogs. It should be emphasized that the same absolute amount of NaCl or saline was infused into obese and lean dogs. Since plasma volume is greater in the obese dogs, the magnitude of the volume expansion was proportionally less in obese than in lean dogs. It is possible that the difference in extracellular volume expansion could have resulted in a difference in the stimulus (increase in heart filling pressure) and this could account for the lesser natriuretic response to acute volume loading in the obese dogs. However, since changes in atrial pressure were not monitored in this experiment, we cannot provide direct evidence that the sodium load resulted in different cardiac filling pressure responses between the obese and lean dogs. Based on our experimental design, we have concluded, however, that for a given sodium load, the obese dogs excrete significantly less sodium than lean dogs and that this acute response is not due to renal nerves.

In summary, we have previously reported that the natriuretic response to an acute saline load is markedly attenuated in obese dogs. We report that the cumulative sodium excretory response to sodium loading is significantly lower in obese dogs. We further demonstrated that there was no difference in the cumulative sodium excretory response between the INN and DNX kidneys of obese dogs. These data indicate that factors other than renal nerves are responsible for the blunted natriuretic response to an acute saline load in obese dogs.

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REFERENCES